Development of gelatin based hydrogel for controlled delivery of anti-microbial .

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Certificate

This is to certify that the thesis entitled "**Development of gelatin based hydrogel for controlled delivery of anti-microbial**" by **SITIPRAGYAN SATAPATHY (212BM1356),** in partial fulfilment of the requirements for the award of the degree of Master of Technology in Biomedical Engineering during session 2012-2014 in the Department of Biotechnology and Medical Engineering, National Institute of Technology Rourkela is an authentic work carried out by him under our supervision and guidance. To the best of our knowledge, the matter embodied in the thesis has not been submitted to any other University/Institute for the award of any Degree or Diploma.

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ABSTRACT

The present manuscript has been designed to study the physicochemical and electrical properties of the gelatin based hydrogel, emulgel and bigel. The chemical studies suggested an increase in the hydrogen bonding in the emulgel and bigel when sesame oil (representative vegetable oil) and sesame oil organogel (representative organogel) were incorporated within the gelatin matrix. Emulgel and bigel showed better mechanical properties and higher electrical impedances as compared to the hydrogel. Hydrogel showed similar swelling at pH 1.2 and pH 7.2. The swelling of the emulgel and bigel was higher in pH 7.2. The formulations were found to be highly hemocompatible indicating their biocompatible nature. Ciprofloxacin, a model antimicrobial drug, was incorporated within the formulations. The release of the drug was found to be diffusion mediated. The antimicrobial efficiency of all the drug loaded formulations was found to be equivalent.

KEYWORDS: Gelatin, Hydrogel, Organogel, Drug delivery, Antimicrobial.

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ABBREVIATIONS

Abbreviation	Definitions
G1	Hydrogel
G2	Emulsion Hydrogels
G3	Bigel
CF	Ciprofloxacin
CPDR	Cumulative percent drug release
DW	Distilled Water
w/w	Weight by Weight
w/v	Weight by Volume
T_m	Melting Point
µm	Micrometer
SR	Swelling Ratio
OD	Optical Density

Chapter 1.

- Introduction

1. INTRODUCTION

Hydrogels have long been utilized for different biomedical requisitions, for example, delicate contact lenses, wound dressing, super-sponges, drug conveyance frameworks and so on . Characteristic polysaccharides are currently widely utilized for the improvement of drug delivey . Polysaccharides are, when all is said in done, non-harmful, biocompatible, biodegradable, and copious. Gels are defined as semisolid formulations. They are usually made up of two components, *viz.* solid (gelator) and liquid (aqueous or non-aqueous) (1). The solid molecules form a 3D network in which the liquid molecules are entrapped (2). If the liquid is aqueous, then the gels are regarded as hydrogels otherwise organogels. In the past decade, there has been an extensive work carried out on hydrogels based systems (3). Hydrogels may be defined as the polymeric architecture having the capability to imbibe and hold water within its structure. The hydrogels have been reported to be mucoadhesive in nature and depends on the composition of the polymer matrix. The mucoadhesive hydrogels helps in delivering drugs to the site of action for prolonged periods. This allows increased bioavailability of the drug (4). Apart from the mucoadhesive property, hydrogels have been reported to alter the release kinetics of the drugs to form controlled delivery matrices by tailoring the crosslinking density of the hydrogels (5). In the past decade, researchers have introduced emulgels (emulsion gels) as controlled delivery vehicles with improved characteristics (6). Emulgels are biphasic systems like emulsions. But the external phase of the emulgels is semisolid in nature, unlike emulsions, which helps in improving the thermodynamic stability of the emulgels (7). This results in the formation of semisolid formulations, having combined advantages of emulsions (controlled release) and gels (thermodynamic stability). Unfortunately, the leaching of the internal oil phase during long-term storage has forced the scientists to look for better formulations, which can be stable during long-term storage (8). This may be attributed to the mismatch in the mechanical

properties of the internal and the external phases. To overcome this problem, recently the concept of bigels has been introduced. Bigels are biphasic systems like emulsions and emulgels but unlike emulsions and emulgels, both the phases (internal and external) are semisolid in nature.

Till date no reports on the comparison of the properties of these three types formulations were found. Taking a note of this, we have tried to develop gelatin based hydrogel, emulgel and bigel and thoroughly characterize their properties. Sesame oil (SO) was used for the development of the emulgels and bigels. It is being obtained from the seeds of *Sesamum indicum*. Span 60 was used as the gelator for sesame oil for the preparation of the organogel (OG; internal phase of bigel) (9). Ciprofloxacin was used as the model antimicrobial drug. The efficacy of the antimicrobial effect of the drug loaded formulations was tested against *E. coli*.

Chapter 2

- Literature Survey
- Objective

Literature Survey :-

Hydrogel is characterized as the water swollen colloidal gel in which the fluid part is water. These are three dimensional structures in which the polymer gets swollen upon imbibitions of vast measure of water. Hydrogel permits free dispersion of a few atoms and the polymer demonstration as a framework to hold water together. Hydrogels can assimilate water or different bio-fluids with some having the capacity to swell. The property of hydrogel incorporates pore size, creation systems, shape and surface/volume proportion, H2o substance, quality and swelling actuation. Because of huge water content in the hydrogel structure it indicates the level of adaptability. Hydrogels are having diverse manifestation of structur. Hydrogels structures are of three sorts macroporous, microporous and non-porous.

Physical and Chemical Properties :-

Solid Weak Condensation Addition Cross-joining

There are two sorts of hydrogels physical and synthetic.

1.1. Physical Hydrogel

Physical hydrogels (Phs) are thermoreversible gel systems made of sub-atomic improvement and optional powers including hydrophobic connections. It is classified into two sorts solid and powerless. Solid gel incorporates polished knobs, lamellar microcrystals and twofold/triple helices. Case incorporates elastomers/ piece copolymers and gelatin. Feeble

gels are because of hydrogen bonds, ionic and hydrophobic acquaintanceships. Case incorporates xanthan, paint and developed acacia gum etc

1.2. Synthetic Hydrogel

At the point when the gels are covalently cross-joined it is called as compound hydrogel. They are likewise termed as lasting hydrogel. The crosslinking thickness and the polymer-water interface are in charge of keeping up the swelling state symmetry of the hydrogels. Concoction hydrogel might be shaped by the expansion of discriminating permeation for instance polyester gel, additionally from buildup for instance polyester gel and structure crosslinking **.**

2. Characterization OF HYDROGELS

There are by and large two sorts of hydrogels.

a. Regular hydrogels

b. Manufactured hydrogels

2. a. Regular hydrogels

Regular hydrogels are existing commonly in nature's turf. These are the material being reviewed for articular tissue building. The polymer utilized within the characteristic hydrogels are common hydrogels are gelatin, methyl cellulose, alginate, agarose/agar, fibrin, chitosan, hyaluronan, chondroitin sulfate and other commonly determined polymers.

Advantages

Because of the incredible biocompatibility regular hydrogels utilized for tissue building requisition. Common hydrogels are additionally having distinctive requisition like they are low harmful repercussions, natural cell co-operations and biodegradable in nature.

Disadvantages

The negative part of common hydrogel incorporates variety of cluster, low mechanical quality and the material determined from creature may pass on infections.

2. b. Hydrogels

Engineered polymer hydrogels constitute a gathering of materials utilized as a part of various biomedical teaches and producing for new guaranteeing requisitions. These hydrogels are combined misleadingly. Produce microarrays and delicate contact lens are produce from engineered hydrogel polymers. Engineered hydrogels have brilliant mechanical properties. Manufactured hydrogels are produced from protein-polymer adducts. The combination of hydrogels was performed through radical copolymerization. For a few cases manufactured hydrogels can perform the undertaking of regular hydrogel.the polymer utilized within the engineered hydrogels are polyanhydrides, poly(aldehyde guluronate), Poly ethylene glycol and so forth.

Hydrogels shows different biomedical requisitions like covering and in gadgets. These hydrogels are having low immunogenicity and minimize the danger of natural pollutions They are having harmful substances and low degradability.

3. Biomedical use of Hydrogels

Hydrogels are used characteristically by the human body, for instance cartilage, mucin, blood clusters and vitreous amusingness of the eye. There is different of requisition Hydrogels like delicate contact lenses, Bioadhesive bearers, Implant coatings, and so forth.

3.1. Use of hydrogels in Drug Delivery

Hydrogels are having different accommodating requisitions in pill conveyance and pharmaceutical sciences because of their extensive measure of water substance. Hydrogels are essentially used for accepted controlled medication discharge framework, bioactive materials and so forth. Hydrogel based pill conveyance framework might be utilized for different sorts like oral, visual, routine, epidermal and subcutaneous requisition. Hydrogels is the suitable medium for the pill conveyance because of its biocompatibility, system structure. Hydrogels are likewise appropriate gene conveyance and subcutaneous conveyance.

3.2. Use of hydrogels in Tissue Engineering

Hydrogels are three dimensional water swollen structures which is insoluble systems of crosslinked hydrophilic polymers. Hydrogels assumes an essential part in diverse tissue designing requisition. Hydrogels have been utilized as framework materials for different purposes like tissue substitution, drug conveyance, call and tissue conveyance, bioactive atom conveyance, space filling executor and different other applications.

3.3. Hydrogels in Biomedical Engineering

Hydrogels have been viably utilized as a part of different biomedical requisitions because of its biocompatible and biodegradable nature[2, 10]. For the biomedical provision the greater part of the polymer utilized for cytotoxicity and within vivo poisonous quality tests. The requisition of hydrogel in biomedical territory holds Phospholipids bilayer, vitality transformation framework, mass transport properties and so forth.

3.4.Hydrogels in Biomaterials

Hydrogels have been utilized as a part of different provisions in biomaterials because of the biodegradable and bioadhesive nature. The case incorporates delicate contact lenses, wound dressing and superabsorbent.

3.5. Use hydrogels in Agriculture

Hydrogel have been utilized as a part of different horticultural requisitions. The water holding ability of the dirt expanded . **Hydrogels** are materials framed by systems of cross-joined hydrophilic polymers that normally hold around 30% in weight of water. Hydrogels have advanced in the course of the most recent decade as materials of decision in differed biomedical provisions. This is connected with the natural biocompatible nature of the hydrogels. The modulationof the properties of the hydrogels is effortlessly conceivable because of the accessibility of polymers of changed science and physical properties. This survey talks about the pharmaceutical parts of the controlled arrival of bioactive executors from hydrogel-based plans. These water-holding gels are as of now the subject of broad research because of their conceivable use on differing and altogether different requisitions, for example, controlled pill discharge, visual gadgets, soil added substance to ration water, wound dressings, nourishment thickening executors, inserts or different provisions that require the usage of biocompatible materials . What's more, some of these hydrogels have been utilized for the improvement of synthetic sensors and, in addition to different utilization, these incorporate particle specific layers, immobilization lattice for the capture of the sensing markers or even as the sensor materials themselves . A gel may be characterized as a 3-dimensional framework of a strong segment, being able to immobilize a fluid part. Contingent upon the miscibility of the fluid part, the gels may be classified either as hydrogels (water-miscible) or organogels (water-immiscible) .The present survey examines the outline systems, instruments of pill discharge, routines for characterization and 30 requisitions of hydrogel-based controlled discharge details. Hydrogel is a 3-dimensional system structure made up of hydrophilic polymers. It can retain and hold water inside its structure. Despite the fact that the hydrogel retains a lot of water, the 3-dimensional system does not get disintegrated. This is either because of the shaping of intersection focuses by

synthetic responses or ensnarement of the polymer chains or vicinity of crystallite spaces connected with the physical cooperations. The polymer framework without the water stage is known as xerogel. At the end of the day, a xerogel is a polymer grid, which when set in a watery media will retain water and experience swelling to structure hydrogel. The absorptionof water by the xerogel is a thermodynamically good process. At the point when a xerogel is set inside water, the water begins to collaborate with the hydrophilic atoms of the polymers. This results in the relocation of water particles into the xerogel and reasons the swelling of the xerogel. This consumed water is viewed as essential bound water As the xerogel guzzle water and swells, the hydrophobic destinations of the polymer chains of the xerogels are uncovered. These hydrophobic spaces likewise associate with water and permit more inflow of water into the lattice. The water retained by the xerogel because of the connection of the water particles with the hydrophobic areas is viewed as auxiliary bound water . The aggregate water guzzled because of the hydrophilic and hydrophobic communications is viewed as aggregate bound water. The xerogels retains water considerably after the hydrophilic and hydrophobic connections because of the osmotic drive. This wonder pushes hydrostatic weight on the polymer grid. The polymer network, thusly, pushes an inverse power to counter the hydrostatic weight. As the hydrostaticpressure and the counter weight by the polymer grid get equivalent, the assimilation of the water into the networks stops . The xerogel is said to achieve the equilibriumswelling. Despite what might be expected, if the polymer lattice is not fit to withstand the hydrostatic weight, the network begins losing its structural honesty and gets broke down in the dissolvable.

a) Hydrogels:

Hydrogels are made up of water dissolvable homopolymers or copolymers and have three dimensional crosslinked organized structures. The hydrogel keeps up its structural and physical honesty inspite of holding water because of the substance and physical crosslink communications that brings about either covalent reinforced or hydrogen fortified structures separately. The vicinity of hydrophilic utilitarian gatherings like O-H, N-H, COO, and so forth in its polymer affix permits the hydrogels to hold substantial measure of water inside it. The water content additionally makes it delicate and rubbery making it helpless in the meantime solid enough to withstand stress like our body tissue. Hydrogels are permeable in nature and discovers provisions in wound recuperating and also bearers in pill conveyance modules for swelling-controlled medication discharge.

b) Organogels.

Organogels are three dimensional crosslinked arranged structures that immobilize natural solvents inside its gelator framework. The grid may be liquid filled lattice or a robust network. The collaboration between the polymer base and lipid base is settled by substances which acts to lessen surface strain and permits their association (compass 60, tween 80). These are semi-strong frameworks and have robust like rheological properties at room temperature.

(C) Emulgels

Emulgels may be characterized as biphasic frameworks including an apolar inner stage (emulsion) inside a fluid gel base. The emulgel framework is a novel methodology for pill conveyance requisitions particularly for hydrophobic pills. The hydrophobic pills is blended in the oil stage which is later consolidated inside the ordinarily steady gel base.

(D) Bigels

Bigels are an alternate class of biphasic crosslinked frameworks that is made up of the oleogels/organogels as the inward stage inside the constant fluid period of hydrogels [12]. Bigels were defined as a novel methodology for creating modules for topical medication conveyance provisions and their expanded bio-adhesion properties assumes a huge part .

OBJECTIVE :-

1. To develop of gelatin based hydrogels, emulgels and bigels.

2. To thoroughly characterize the above formulations by physicochemical, mechanical, electrical and biological activity studies.

Chapter 3.

- Materials and Methods.

3. MATERIALS

Gelatin and Tween 80 (polyxyethylene sorbitan monooleate) were procured from Himedia, Mumbai, India. Ethanol was obtained from Honyon International Inc., Hong Yang Chemical Corpn., China. Glutaraldehyde (25%, for synthesis; GA) and hydrocholric acid (35% pure) was obtained from Merck Specialities Private Limited Mumbai, India. Span 60 was procured from Loba chemie, Mumbai, India. Sesame oil (Tilsona®, Recon Oil Industries Pvt. Ltd., Mumbai, India) was obtained from the local market. Goat intestine and blood were obtained from local butcher shop. Double distilled water was used throughout the study. Ciprofloxacin was procured from Fluka, China. *E. coli* (NCIM 2563) was purchased from NCIM, Pune, India.

3.2 METHODS

3.2.1 Preparation of hydrogel

Twenty percent (w/w) of gelatin solution (GS) was prepared by dissolving 20 g of gelatin in 70 g of water, whose temperature was maintained at 70 °C and kept on stirring at 600 rpm to obtain a clear homogenous solution. The final weight of the gelatin solution was made to 100 g.

3.2.2 Preparation of emulgels

Emulgel was prepared as per the protocol reported earlier (10). In brief, 2.5 g of sesame oil was slowly added to a mixture of 0.5 g of Tween 80 and 17. 5 g of previously prepared gelatin solution, maintained at 70 ^{o}C (600 rpm, magnetic stirrer). The stirring was done for 15 min for proper homogenization. 0.5 ml of glutaraldehyde reagent (2 ml Glutaraldehyde + 2 ml ethanol + 0.05 ml of 0.01 N hydrochloric acid) was added to the above mixture, mixed for 10 sec (600 rpm, 70 ^{o}C) and immediately poured in either petriplates or cylindrical moulds.

The petriplates/moulds were incubated at 37 ^{o}C for 30 min to induce gellation and formation of emulgel.

3.2.3 Preparation of organogel

Sesame oil organogel was prepared by dissolving 1.5 g of span 60 in 8.5 g of sesame oil, maintained at 70 ^{o}C (100 rpm) (11). The hot homogenous solution, so formed, was kept under room temperature (25 ^{o}C) to form organogel.

3.2.4 Preparation of bigel

Bigel was prepared as per the methodology described for emulgels with slight modification. Sesame oil based organogel, maintained at 70 ^{o}C, was used instead of sesame oil for the preparation of the bigel. Rest of the procedure remained same. .

3.2.5 Preparation of drug loaded samples

Drug loaded hydrogels, emulgel and bigel were prepared in a similar manner. Ciprofloxacin was dispersed in gelatin solution for preparing the drug loaded hydrogel(12). The drug was dispersed in sesame oil and sesame oil organogel for preparing drug loaded emulgel and bigel, respectively. The final concentration of ciprofloxacin in the formulations was 1 % (w/w). The compositions of the formulations have been tabulated in Table 1.

Table 1. Compositions of the formulations developed

Sample code	GS (g)	SO (g)	OG (g)	Tween 80 (ml)	Ciprofloxacin (g)
G1	20	--	--	--	--
G1C	19.8	--	--	--	0.2
G2	17.5	2.5	--	0.5	--
G2C	17.3	2.5	--	0.5	0.2
G3	17.5	--	--	0.5	--
G3C	17.3	--	2.5	0.5	0.2

3.2.6 SEM

The surface morphology of the formulations was studied under field emission scanning electron microscope (NOVA NANO/SEM) after converting them into xerogels. The formulations were dried for 48 h (40 ^{o}C) to convert the formulations into xerogels. The xerogels were sputter coated with platinum before analysis.

3.2.7 Molecular interactions

The formulations were cut into pieces of 1 cm x 1cm and were analysed using X-ray diffractometer (XRD-PW 1700, Philips, Rockville, USA).The x-ray radiation source was Cu-Kα and was being operated at 30 kV and 20 mA (14). The scanning was done in the diffraction angle ranges of 5° -50° 2θ at a rate of 2° 2θ per min.

The chemical interactions amongst the components of the formulations were studied using Fourier Transform Infrared Spectroscope (FTIR; Alfa-E, Bruker, Germany) working in the ATR-mode. The scanning was done in the wavenumber range of 500-4000 cm^{-1} (15) .

3.2.8 Swelling studies

The swelling profile of the formulations were checked at acidic (HCl buffer, pH=1.2) and basic (Phosphate buffer, pH= 7.2) conditions. The formulations were cut into pieces of 1 cm x 1cm. The pieces were weighted accurately (Wi) and immersed in 50 ml of buffers. The samples were taken out of the buffers at regular intervals of 30 min for first 1 h and 1 h thereafter for 7 h (16). The surface moisture of the formulation pieces was removed using Whatmann paper and weighed accurately (W_t). The % of swelling ratio was calculated by the equation:

$$\% \text{ swelling} = \frac{W_t - W_i}{W_i} * 100$$

where,

W_i= Initial weight of the gel

W_t= Weight of the swollen gel

3.2.9 Mucoadhesion studies

Mucoadhesive properties of the formulations were tested using wash-off method and mechanical tester. Goat intestinal mucosa was used for the study. Mucoadhesion using wash-off method was conducted in a tablet disintegration apparatus. The intestinal lumen was cut open and attached to glass slides, such that the intestinal mucosa is exposed outwards, using commercially available acrylate adhesive. Formulations (5 mm x 5 mm) were put on the exposed surface of the intestinal mucosa. A weight of 5 g was applied over the formulations for 5 min. Thereafter, the slides were vertically put into USP disintegration baskets. Phosphate buffer (pH=7.2) was used as the disintegration medium. The experiment was carried out for 24 h. The setup was continuously monitored to detect the detachment of the formulations from the intestinal mucosal surface. The time required to detach the formulations from the mucosal surface was noted down (17).

3.2.10 Mechanical Properties

The mechanical properties of the formulations were studied using texture analyzer. The mechanical properties of the formulations were studied by performing a series of tests (compression) at room temperature. The experimental parameters have been listed in table 2 (20).

Table 2. Instrumental parameters for texture analyzer

Type of study	**Type of fixture**	**Testing conditions**			**Mode of study**
		Pre test speed (mm/sec)	**Test speed (mm/sec)**	**Post test speed (mm/sec)**	
Compression	HDP/SR spreadability rig with 45° conical perspex probe P/3 30mm Diameter cylindrical probe	1	0.5	10.0	Auto force (20 g, 5 mm)

3.2.11 Drug release studies

In vitro release study of ciprofloxacin was carried out as per the reported literature with slight modifications (3). In short, circular pieces of formulations (1.9 cm diameter) were immersed in 50 ml of dissolution media, kept on stirring at 100 rpm (37 ^{o}C). The dissolution media was HCl buffer (pH= 1.2) for the first 2 h and phosphate buffer (pH 7.2) for the next 7 h. At regular intervals of time, the dissolution media was replaced with fresh dissolution media to maintain the sink conditions. The replaced dissolution media was analyzed spectrophotometrically at 271 nm using UV-Visible spectrophotometer to determine the concentration of ciprofloxacin (23).

The efficacy of the formulations to release the drug in its active form and inhibit the growth of the microbes was tested by agar diffusion method. 100 µl of *E. coli* suspension (2 x 10 ^{5} CFU/ml) was spread over the nutrient agar plates. The pieces (7 mm diameter) of the formulations (with and without ciprofloxacin) were put over the agar plates. The agar plates were subsequently incubated at 37 ºC (12 h). The zone of inhibition of the microbe was measured using a ruler at the end of the study.

3.2.12 Biocompatibility

The preliminary biocompatibility test of the formulations was estimated by hemocompatibility test as per the previously reported literature (24-25). In short, citrated goat blood was diluted with normal saline (4:5 ratio). The formulations were cut into pieces of 1 cm x 1 cm. The pieces were poured into falcon tubes. 0.5 ml of diluted blood was added to the falcon tubes followed by sufficient amount of normal saline to make up the final volume

to 10 ml. Positive and negative controls were prepared by taking 0.5 ml of 0.01 N HCl and 0.5 ml of normal saline, respectively. The falcon tubes were then incubated at 37 ºC for 60 min. The falcon tubes were centrifuged at 3000 rpm for 10 min. The optical density of the supernatant was measured at 545 nm using UV-Visible spectrophotometer. The % hemolysis was calculated as per the following formula (26).

$$\%\text{Hemolysis} = \frac{\text{O.D.test} - \text{O.D.negative}}{\text{O.D.positive} - \text{O.D.negative}} \times 100$$

where,

OD_{test}= optical density for the test sample

$OD_{positive}$= optical density for the positive control

$OD_{negative}$= optical density for the negative control

The biocompatibility of the prepared formulations was evaluated using HaCaT cell line by MTT assay following protocol described elsewhere (27). The leachants of the formulations were prepared by incubating 1 g of the formulations in 30 ml of PBS for 24 h at 37 ^{o}C. The supernatant was used for the analysis (28). The cells were seeded into a 96 well plate at a cell concentration of 1 X 10^4 cells /well and incubated at 37 ºC in CO_2 for 24 h. Thereafter, 20 µl of the leachants was added to each well and incubated for 24 h. The cell viability was estimated using MTT assay after 24 h.

3.2.13 Leaching studies

Initially, the leaching of the internal phase from the formulations were checked qualitatively using filter paper method described elsewhere[13]. In brief, the formulations were cut in circular slabs of diameter 1.9 cm. The slabs were kept on Whatmann filter paper and subsequently incubated at 25 oC for 3 h. The leakage of the internal phase, if any, appears as a dark shaded region around the formulations . The diameter of the dark zone gives information about the leakage of the internal phase.

The leaching of the internal phase was quantitatively measured as per the reported literature[14]. Accurately weighed 0.1 g (w1) of the formulations was soaked in 1.0 g of ionized water for 30 min at 25 ºC in 2 ml eppendorf tubes. The whole content was centrifuged at 1000 rpm for 2 min. The precipitate and the supernatant were weighed and separately dried in a ventilated oven at 55 °C for 48 h. The dried supernatant (w2), was weighed again.

The percentage of leaching was calculated as:

Percentage of leaching =w2x100/w1.

Chapter 4.

➢ Results and Discussion

4 RESULTS AND DISCUSSIONS

4.1 Preparation of hydrogels

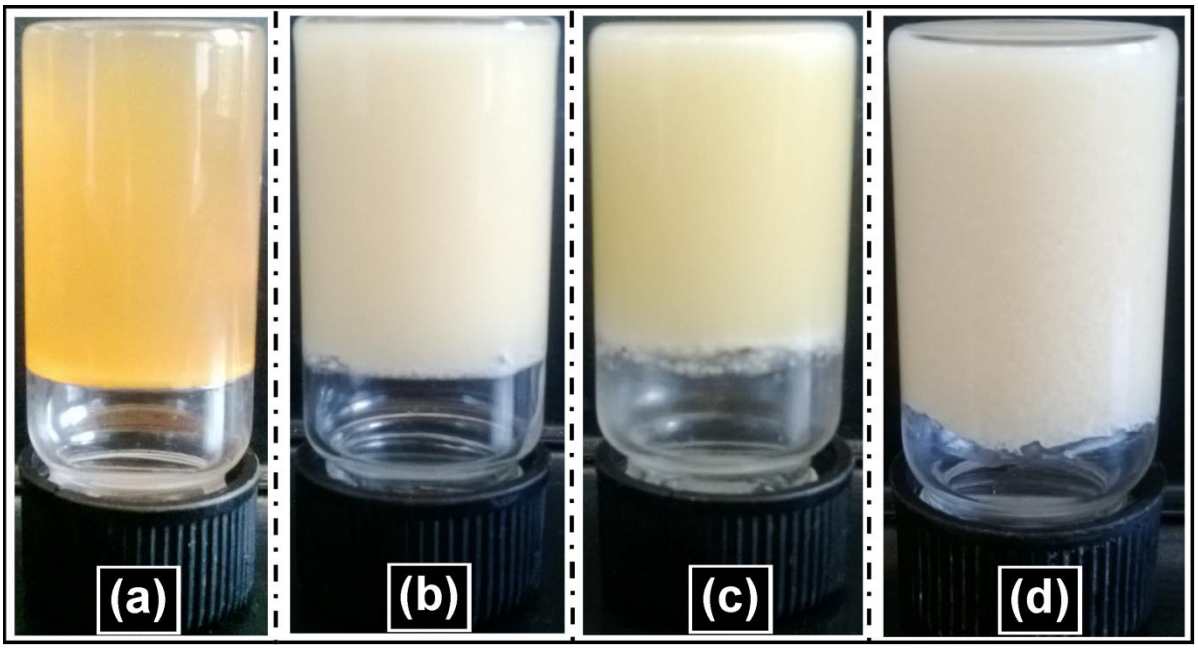

Fig 1. Preparation of hydrogels (a) Hydrogel , (b) Emulgels ,(c) Organogel , (d) Bigel

The pictographs of the gelatin hydrogels, emulgel and bigel has been demonstrated in Figure 1. Gelatin hydrogel was light caramel yellow in shade and translucent. The emulgel and bigel were hazy. Emulgel was light caramel white in color. The bigel was somewhat darker than the emulgel (dim borwnish white). The whitish tinge of the emulgel and the bigel may be clarified by the diffraction of light from the interface of the immiscible stages (a property regularly connected with the emulsions) (29). All the details had a smooth composition and had an alleviating impact.

4.2 SEM :-

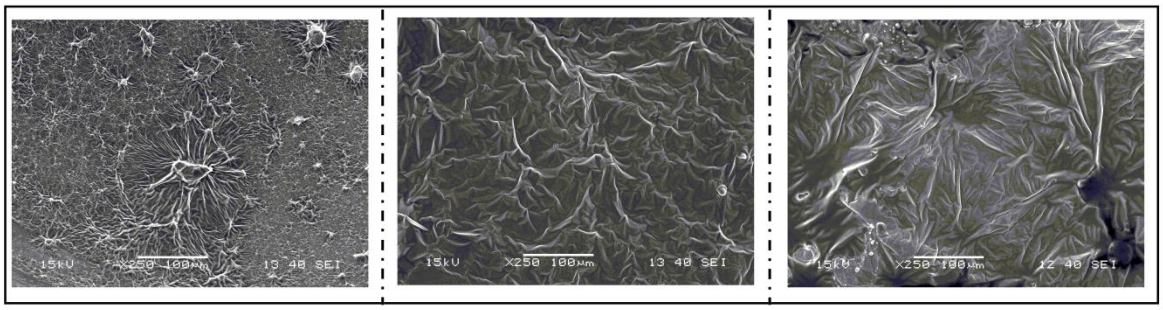

Fig 2. SEM of (d) Gelatin, (e)Emulgel and (f) Bigel.

The microstructure of the gelatin smears did not indicate any clear structural engineering. The emulgel and the bigel demonstrated vicinity of agglomerated globular structures scattered consistently all through the gelatin framework. The agglomerated scattered stage in bigel was sporadic fit as a fiddle (30).

The surface topology of the definitions were mulled over under field emmision filtering electron magnifying lens in the wake of changing over the plans into xerogels. The hydrogels demonstrated a smooth surface. This was because of the nonattendance of any inside structures. Emulgel and bigel demonstrated the vicinity of agglomerated globular structures scattered inside a polymer continuum stage. Separated from the agglomerated particles, fiber like structures were additionally noticeable in bigel. The micrographs of the plans from light microscopy and SEM were in backing of one another((31)

4.3 Molecular interactions

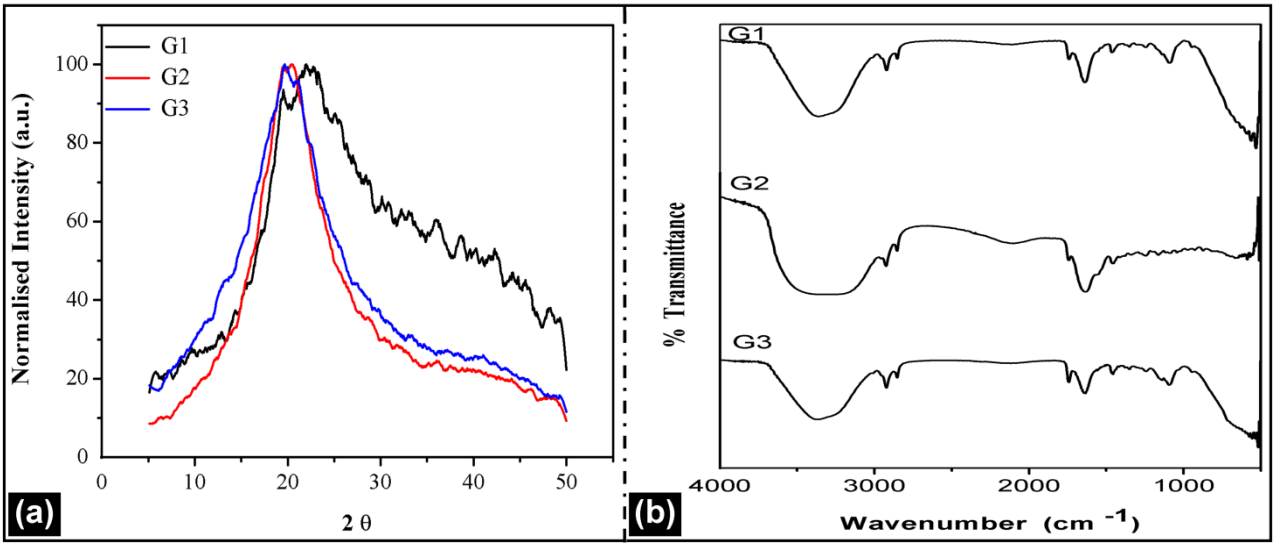

Fig 3. Molecular interactions, (a) XRD profile, and (b) FTIR profile of the formulations

The crystalline natures of the gelatin were additionally affirmed by investigation of the XRD design. Fig. 2 shows the XRD example of the gelatin and gelatin . It appears that the control film did not uncover any diffraction crest for silver, while the gelatin/Agnps film uncovered four different diffraction crests at 38.2°, 44.4°, 64.4°, and 78.2° of the 2θ.These common XRD crests demonstrate the shaping of a face focused cubic (fcc) structure of the crystalline. Among the comparing planes, plane showed a higher force than alternate planes, which may be because of the overwhelming introduction of the plane. The power of the diffraction tops differed with expanding amassing of the Agno3 added to the gelatin movies. Furthermore, an unidentified crest was watched for both control and gelatin/Agnps movies at 28.2° which came about because of the polymer gelatin**.** A FT-IR spectroscopy estimation was additionally done to investigate the conceivable practical gatherings of gelatin in charge of diminishment and adjustment of the Agnps. Fig. 3 shows the FT-IR spectra of the gelatin and gelatin/Agnps (40 mg) nanocomposite movies. As demonstrated in Fig. 3, both control and

gelatin/Agnps movies uncovered different trademark tops running from 3288 cm−1 to 664 cm−1. An expansive and solid assimilation crest was seen at 3288 cm−1, which demonstrates the extending vibration of the hydroxyl bunches (O–h). Pandey et al. (2012) found that the O–h gathering of polymer had productive coordination capability with silver particles. The trademark ingestion top showing up at 2933 cm−1 relates to the C–h extending recurrence. The extreme crest at 1631 cm−1 demonstrates the vicinity of the carbonyl (Cdouble security; length as m-dasho) extending recurrence (Pereda et al., 2011). The crests showing up at 1551 cm−1 are because of the extending of N–h (amide II). Crests from 1451 to 1239 cm−1 demonstrate C–n and N–h extending vibrations (amide III) (Yin, Li, Sun, & Yao, 2005). All tops saw in the FT-IR spectra of the control and gelatin/Agnps demonstrate moderately comparative examples with the exception of force of the crest tallness. What's more, no extra top development was seen in the FI-IR range of the gelatin/Agnps movies, proposing that no compound bond shaping happene

The normalized x-ray diffractograms of the hydrogel, emulgel and bigel showed a broad peak at ~20^{o} 2θ. This kind of XRD profile is generally associated with the amorphous formulations. Full-width at half-maximum (FWHM) of the peaks of the diffractograms was calculated from the XRD profile (Table 1). The FWHMs were found to be in the order of G1> G3 > G2. Higher FWHM values suggest lower crystallinity (or higher amorphosity) (32). The results indicated that G2 was having higher crystallinity as compared to G3 followed by G1. The higher crystallinity of G2 and G3 as compared to G1 can be explained by the higher degree of hydrogen bonding of the gelatin molecules with the fatty acids, present in sesame oil and sesame oil organogels. G3 showed intermediate crystallinity due to the availability of the lesser number of fatty acid molecules (as compared to G2) for hydrogen bonding due to the interaction with span 60. The XRD examples of the GP

also pectin showed that there was lessening in crystallinity of pectin when it was altered with polyacrylamide. Likewise, the XRD example of the GP was completely transformed from that of pectin demonstrating the development of another item. From the XRD plots the % crystallinity of pectin and joining copolymer was discovered to be 0.81 and 0.69 separately. This lessening in crystallinity with copolymerization is may be because of joining of bulkier gathering inside the polymeric system, which thusly diminishes intermolecular hydrogen holding. Since no fatty acid was present in G1, the degree of hydrogen bonding was lowest and hence the crystallinity. Similar results were also obtained by FTIR studies reported below.

Table S1 Molecular properties of the formulations

Fomulation	FWHM (from XRD)	AUC (from FTIR)
G1	24.2	802.12
G2	9.25	522.01
G3	11.58	607.96

FTIR studies of the formulations were conducted to understand the interactions amongst the functional groups. The peak at ~1640 cm^{-1} may be associated with the C=O stretching of the amide-I bands. The peak at ~1540 cm^{-1} may be explained by the presence of amide-II bands. The peak at ~3400 cm^{-1} was due to combined O-H and N-H stretching vibrations. The formation of broad peak at the said region suggested presence of intermolecular hydrogen bonding. The absorption peaks in the region of 3000–2800 cm^{-1} was due to C–H stretching

vibrations (33). The stretching vibrations of methylene (–CH_2–) and methyl (–CH_3) groups were observed at ~2920 cm^{-1} and ~2850 cm^{-1}, respectively. examined . Gelatin dissolving and discovered it to be connected with diminishment in the 1678 cm −1 crest and 1660/1690 cm−1 top power proportion and build in amide I parts happening around 1613, 1629 and 1645 cm−1. These creators allocated the groups happening at 1645–1657 cm−1to arbitrary loops and the 1660 cm−1band to triple helix, with commitment from α-helix and β-turns. The amide I part, at 1690 cm−1, has been credited to helices of total collagenAll the peaks of the gelatin hydrogel were conserved in the emulgel and the bigel. The FTIR spectra of the un-adulterated pectin.

The range indicated top at 3415 cm–1 because of the extending of –oh gatherings. The crests at 2913 cm–1.Diagrammatic representation of Franz's dissemination cell showed –c–h extending vibration. The crest at 1756 cm–1 showed c = O extending vibrations because of the vicinity of –cooch3 bunch. The crests at 1441 cm–1 and 1342 cm–1 could be appointed to –ch2 scissoring and –oh curving vibration, separately. The crest at 1023 cm–1 proposed –ch–o– Ch– extending. The crest at 1150 cm–1 proposed the vicinity of –ch. The peak at ~3400 cm^{-1} was broadened in the emulgel and the bigel. This was due to the higher degree of hydrogen bonding amongst the fatty acid and gelatin molecules. No peaks corresponding to ciprofloxacin was observed in the drug loaded formulations (Figure 3). This may be explained by the presence of the drug in very minute concentration and hence the peaks due to the drugs were completely suppressed by the strong peaks of the gelatin hydogel matrices.

4.4 Swelling Studies

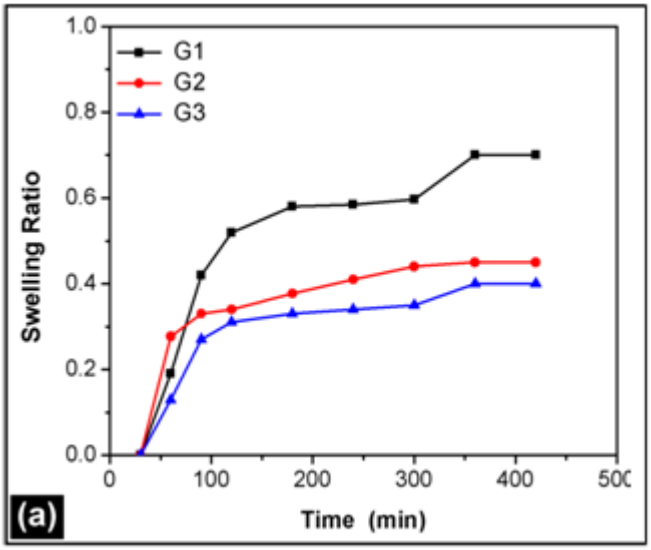

Fig 4. Swelling study (a) pH 1.2

The swelling profile of the formulations were checked in pH= 1.2 and pH= 7.2. G1 showed nearly equal swelling in both the acidic and baisc conditions. This may be due to the presence of both anionic (COO^-) and cationic (NH_4^+) groups which are present in gelatin. G2 and G3 showed lower swelling in acidic pH as compared to the basic pH. This was due to the presence of fatty acid molecules (in sesame oil). The fatty acids did not accommodate the aqueous phase within its structure at lower pH due to the electrostatic shielding effect (34). On the other hand, the electrostatic shielding effect was lower at pH 7.2 and hence allowed accommodation of the aquesous phase. The accommodation of the aqueous phase was limited by the elastic force exerted by the gelatin matix.

Initially, the leaching of the internal phase from the formulations were checked qualitatively using filter paper method described elsewhere[13]. In brief, the formulations were cut in circular slabs of diameter 1.9 cm. The slabs were kept on Whatmann filter paper and subsequently incubated at 25 oC for 3 h. The leakage of the internal phase, if any, appears as a dark shaded region around the formulations . The diameter of the dark zone gives information about the leakage of the internal phase.

The leaching of the internal phase was quantitatively measured as per the reported literature[14]. Accurately weighed 0.1 g (w1) of the formulations was soaked in 1.0 g of ionized water for 30 min at 25 ºC in 2 ml eppendorf tubes. The whole content was centrifuged at 1000 rpm for 2 min. The precipitate and the supernatant were weighed and separately dried in a ventilated oven at 55 °C for 48 h. The dried supernatant (w2), was weighed again.

4.5 Mucoadhesive properties

The mucoadhesive properties of the formulations were tested by *in vitro* wash-off method and mechanical testing method. The wash-off method deals with the determining the adhesion time of the formulations with the mucosal surface while the interface is being moved in and out of the media. The test was done in phosphate buffer (pH 7.2). The detaching time of G1, G2 and G3 was found to be 1200 ± 20 min, 1080 ± 15 min, 1140 ± 30 min, respectively. The results indicated that the mucoadhesivity of G1 was highest followed by G3 and G2, respectively. The encapsulation of the oil and organogel has reduced the mucoadhesive properties. Similar results have been reported earlier when oils and organogels have been encapsulated within polymeric microparticles (35). The decrease in the mucoadhesive property has been explained by the leaching of the internal phase, which alters the interaction between the formulation and the mucosal surface.

The work done to separate the formulations from the mucosal surface is regarded as the work of adhesion. The work of adhesion is generally calculated from the area under the curve

(AUC) of the force-time (force-distance) profile. The AUC was found to be in the order of G1 > G3 > G2. The results are in accordance with the wash-off test (36).

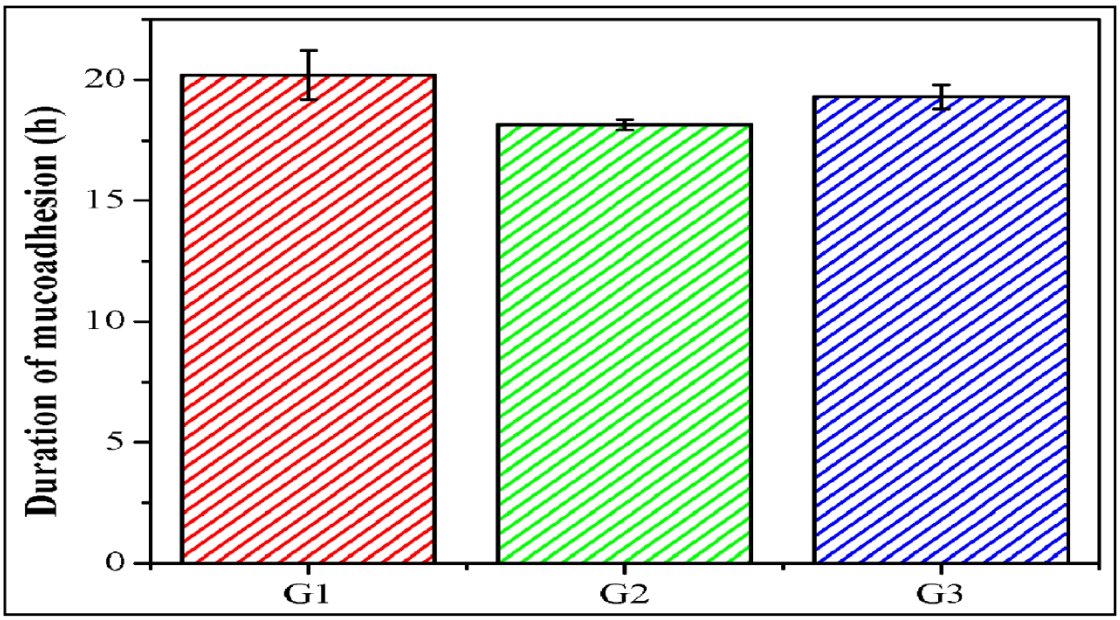

Fig 6. Mucoadhesive property (a) Wash-off method,

4.6 Mechanical Properties

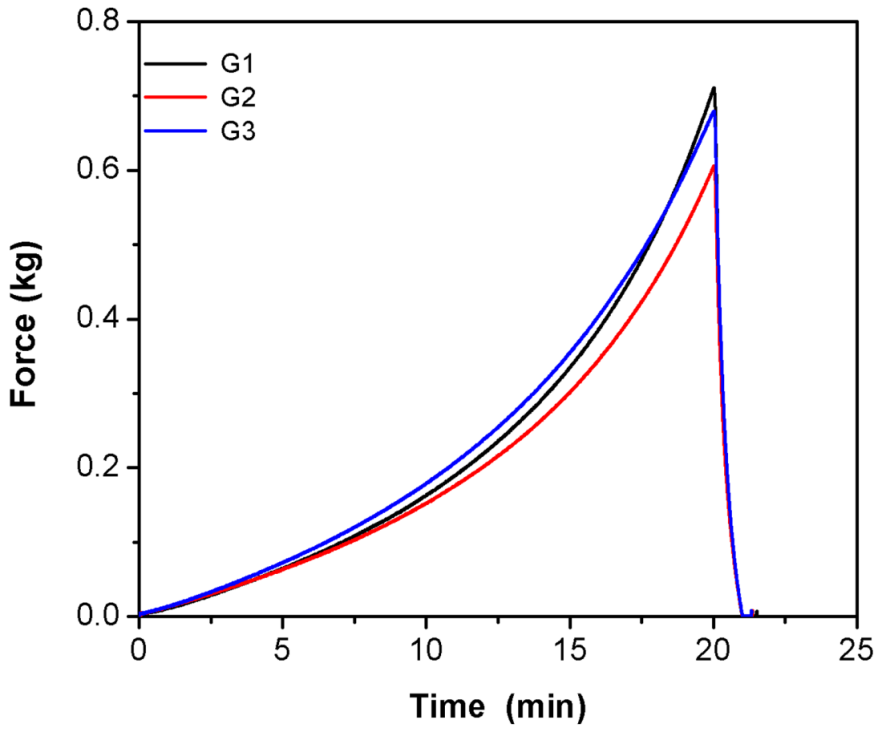

Fig7. Mechanical (a)Compression (0.5 mm/sec)

Sample	Maximum Load (kg)	Work of shear (kg.sec)
G1	0.711	0.878
G2	0.606	0.844
G3	0.680	0.976

The textural properties of the naturally ready gels were examined by performing an arrangement of tests. The qualities of the gels were dead set utilizing a 3 mm SS barrel shaped test. The test was permitted to enter into the gels at a rate of 0.5 mm/sec. The study gave an evidence about the hardness, inflexibility, fragility, stickiness and adhesiveness of the gels . The results proposed a decline in the hardness, fragility and inflexibility of the gels with an ensuing build in the G2 extent in the gels .The study recommended a lessening in the mechanical quality of the gels with the build in the G2 proportion. The demonstrated better

quality as contrasted with the uehs. This may be ascribed to the change of the gel properties because of the crosslinking of the gelatin frameworks .

The gels were twisted by permitting the test to move to a set separation 5mm) after a trigger energy of 20 g. The test was kept at the position for a time of 60 sec and a comparing diminishment in the anxiety was recorded . The different textural properties were computed. The aftereffects of the study recommended that there was a decline in the energy needed to infiltrate the target separation with a build in the G2 extent in the gels. This proposed that the gels, having high solidness as contrasted with the others and there was a reduction in the immovability of the gels as the extent of the G2 was expanded. The solidness of the gels was likewise affirmed byascertaining .. It was discovered that the test moved less separation for gels contrasted with the others. This additionally recommended that these gels were having higher mmovability. The immovability of the gels was brought down with the build in the G2 extent. The F0 also F30 strengths were dead set to discover the unwinding and % unwinding of the gelled structures the gels uncovers data about the capacity of the gels to misshape when an anxiety (energy) is connected and is regularly spoken to by modulus of deformability (MD). A reduction in the WR and the % unwinding with the build in MO extent proposed that the gels holding higher measure of MO were relaxed to a more terrific degree when anxiety was connected. A decline in the MD in the gels with higher MO substance proposed that the gels were diminished with the expansion of oil.time of time/separation and along these lines withdrew. A shut hysteresis circle proposed aggregate compromise of the beginning microstructure as the anxiety is releaved. The territory of the hysteresis circle was computed and demonstrated. Hysteresis circle territory means the measure of work done to recover the introductory structure 49. With the expand in the MO extent, there was a diminish in hysteresis circle region. This proposed that the gels were

getting less thick and thus a less measure of work was required to recover the introductory structure

4.7 **Drug release Studies**

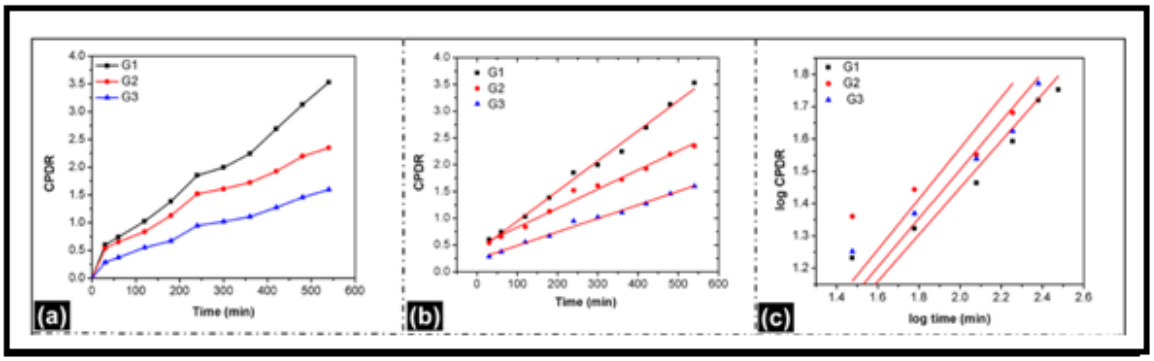

Fig 10 . Drug Release studies (a) drug release profile, (b) zero order release model, (c) KP diffuision model.

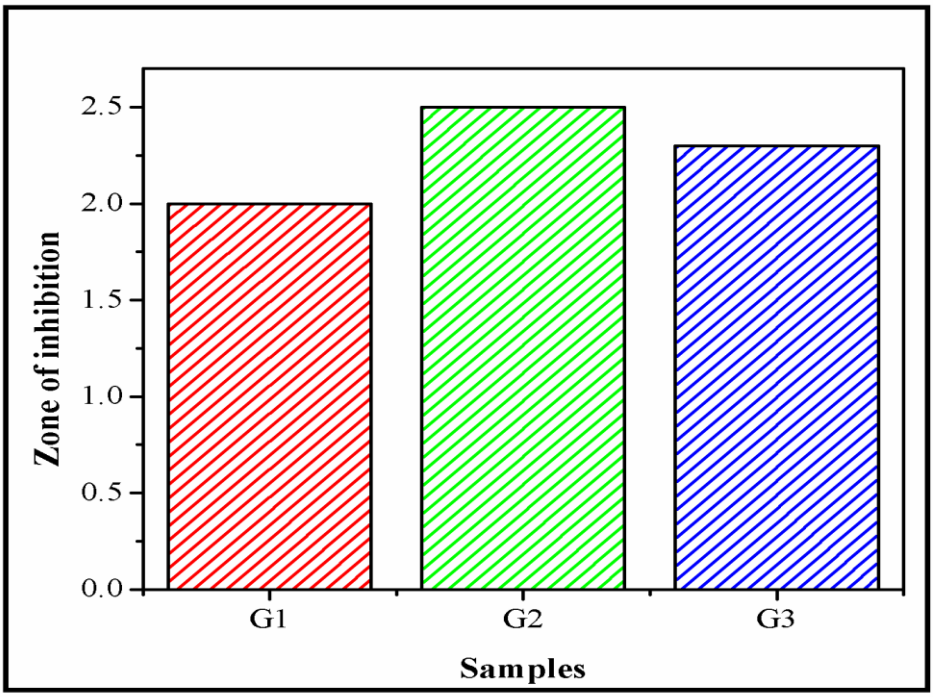

Fig11 .Antimicrobial efficency of (d) G1, (e) G2, and (f)G3.

The *in vitro* drug release profiles of ciprofloxacin from the formulations have been shown in Figure 10. The release study was carried out at pH 1.2 for 2 h followed by at pH 7.2 for 7 h. At the end of the study, G1 showed highest cumulative percentage drug release (CPDR) followed by G2 and G3, respectively. The trend of the release of the drugs was in direct relation with the electrical conductivity and swelling behaviour of the formulations (43). The release kinetics of the drugs was estimated by fitting various models, viz. zero-order, first-order and Higuchi models. The correlation coefficient values of the model fittings suggested that the best-fit model was zero-order kinetics. Zero-order kinetics model is shown by the reservoir type delivery vehicle. Hence the developed formulations may be regarded as reservoir-type delivery vehicle. The n-value was calculated from the KP model. The results suggested that G2C showed Fickian-diffusion whereas G1C and G3C showed non-Fickian-diffusion mediated drug release (44).

Table: drug release studies of the formulations

Samples	CPDR	Zero Order	KP Model		Type Of Release
		R^2	R^2	n-value	
G1C	3.52	0.991	0.999	0.503	Non-Fickian Diffusion
G2C	2.34	0.983	0.957	0.396	Fickian Diffusion
G3C	1.59	0.991	0.999	0.501	Non-Fickian Diffusion

The qualitative drug release study from the formulations were studied by determining the antimicrobial efficiency of the formulations against *E. coli*. The zone of inhibition (indicator of antimicrobial efficiency) was found to be similar in all the formulations.

4.8 Biocompatibility

The preliminary biocompatibility of the formulations were tested by hemocompatibility test. The test aims at measuring the percentage of red blood cells damaged (% hemolysis) in the presence of the formulations. The damaged red blood cells release hemoglobin to the aqueous continuum phase, which in turn, results in the yellowish coloration. The falcon tubes are centrifuged and the supernatant is collected (45). The yellowish color is measured spectrophotometrically in the supernatant. Higher the optical density of the supernatant, higher is the cell damage. The % hemolysis in the presence of the developed formulations were found to be < 5% (Figure 11). This suggested the probable biocompatible nature of the developed hydrogels. The proliferation index of the HaCaT cells in the presence of the leachants has been shown in (Figure 11). The variations in the proliferation indices of the formulations were found to be statistically insignificant ($p < 0.05$) as compared to the control. Also, there were no visual differences in the morphology of the cells in the presence of the leachants. This suggested that the leachants did not have any detrimental effect on the proliferation of the cells thereby indicating the biocompatible nature of the prepared formulations.

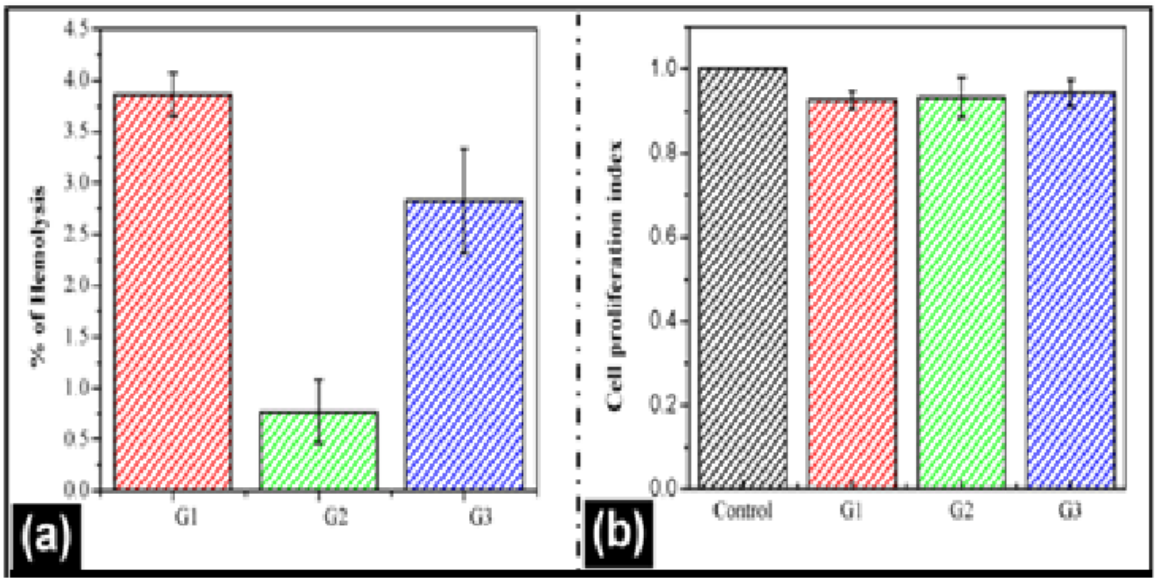

Fig11. Biocompatibility studies (a) hemocompatibility, (b) HaCaT cell viability index and cell morphology.

CHAPTER 5

- **CONCLUSION :-**
- **ACKNOWLEDGMENT**
- **REFERENCES**

CONCLUSION :-

The present study describes the comparison of the properties of the gelain based hydrogel, emulgel and bigel. XRD and FTIR studies suggested that the incorporation of the sesame oil and sesame oil organogels within the gelatin matrix resulted in the increase in the crystallinity. This resulted in the increase in the mechanical properties of the emulgel and the bigel. The impedance of the emulgel and the bigel was higher . The swelling index was lower in the emulgel and bigel. The drug release from the formulations was found to be diffusion mediated. The formulations showed sufficient antimicrobial properties to be used for delivery of antimicrobial drugs.

ACKNOWLEDGMENT

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